Recent developments in ex vivo platelet production

Retno Wahyu Nurhayati · Yoshihiro Ojima ·
Masahito Taya

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Abstract The platelet is a component of blood that functions to initiate blood clotting. Abnormal platelet count and function can lead to a life-threatening condition caused by excessive bleeding. At present, platelet supply for transfusion can be obtained only from platelet donation. However, platelets cannot be stored for longer than 7 days, meaning that routine isolation is required to maintain platelet supply for transfusion. To mitigate for potential platelet shortages, several strategies have been proposed to generate platelets ex vivo. By employing both of natural and artificial approaches, several researchers have successfully generated biomaterials with characteristics similar to human-derived platelets. Their reports indicated that the biomaterials could mimic the aggregation of human-isolated platelets, further suggesting the possibility to substitute or complement human-isolated platelets. The current review summarizes the progress in ex vivo platelet production and gives a prospect for the possible approaches to

achieving a feasible platelet factory, based on the Good Manufacturing Practice standards.

Keywords Platelets · Megakaryocytes · Megakaryopoiesis · Ex vivo production · Immortalized cells

Introduction

The platelet is a component of blood with a primary function of maintaining the integrity of blood vessels through a blood clotting mechanism. Resting platelets circulate inside of blood vessels as disc-shaped fragments of about 3 μm in diameter (Nachmias and Yoshida 1988) and are activated upon contact with exposed collagen and agonists released by injured blood vessels, such as thrombin, thromboxane A₂ (TXA₂), and adenosine diphosphate (ADP), causing a massive change in platelet morphology (Gresele 2008). The activated platelets subsequently stimulate platelet glycoproteins, including GPIb and GPIIb/IIIa, and release several active molecules from granules, including ADP and TXA₂ (Hoffbrand et al. 2011), to recruit more platelets (Fig. 1a). Platelets play a critical role in homeostasis: patients with a deficient number or abnormality of platelets often experience excessive bleeding (Pendaries et al. 2007), which could lead to a life-threatening condition.

In the human bone marrow (BM), platelets are produced from multipotent hematopoietic stem cells (HSCs) through a unique cellular developmental

R. W. Nurhayati (✉) · Y. Ojima · M. Taya
Division of Chemical Engineering, Department of
Materials Engineering Science, Graduate School of
Engineering Science, Osaka University, 1-3
Machikaneyama-cho, Toyonaka, Osaka 560-8531, Japan
e-mail: retno@cheng.es.osaka-u.ac.jp;
nretnowahyu@yahoo.com

Y. Ojima
e-mail: ojima@cheng.es.osaka-u.ac.jp

M. Taya
e-mail: taya@cheng.es.osaka-u.ac.jp

Fig. 1 **a** Platelet activation characterized by platelet–vessel wall interaction (adhesion) and platelet–platelet interaction (aggregation). **b** Megakaryopoiesis of an HSC for generating platelets in the bone marrow (BM). *ADP* adenosine diphosphate, *CD* cluster of differentiation, *GP* glycoprotein, *HSC* hematopoietic stem cell, *MK* megakaryocyte, *TXA2* thromboxane A2, *vWF* von Willebrand factor

process called megakaryopoiesis (Fig. 1b). HSCs are the progenitor cells for all blood cell types including leukocytes, erythrocytes and platelets (Akashi et al. 2000) and can be specifically directed into the megakaryopoiesis upon interaction with thrombopoietin (TPO) (Deutsch and Tomer 2006). In addition to TPO, various cytokines have been identified as stimulating factors during megakaryopoiesis, including interleukin (IL)-3, IL-6, IL-9 (Chen et al. 2009a, b), IL-11 and stem cell factor (SCF) (Teramura et al. 1992; Panuganti et al. 2013). Although the absence of these cytokines does not prevent the progress of TPO-induced megakaryopoiesis, the addition of cytokines has been reported to stimulate megakaryopoiesis (Deutsch and Tomer 2006; Panuganti et al. 2013). SCF plays an important role for increasing cell proliferation in early megakaryopoiesis (Chang et al. 2007). IL-3 has a synergistic effect with TPO on promoting megakaryopoiesis, whereas the benefit of IL-6 or IL-11 remains unclear (Panuganti et al. 2013).

The stages of megakaryopoiesis can be identified from the expression profiles of surface markers, including CD34, CD41, CD42b, von Willebrand Factor (vWF) and glycoprotein V (GPV) (Chang et al. 2007). CD34 is expressed in megakaryocyte (MK) progenitors in a manner of gradual decrease during megakaryopoiesis. The cells differentiate into megakaryoblasts, which is characterized from the increase of CD41 (GPIIb/IIIa) expression (Deutsch and Tomer 2006). CD41 is expressed throughout megakaryopoiesis and is thus considered as a universal marker for MKs. In the next stage, megakaryoblasts undergo polyploidization to generate polyploid MKs. CD42b (GPIb) is detected in the late-stage of megakaryopoiesis and becomes an indication of MK maturation, along with GPV and vWF expression (Chang et al. 2007).

A unique characteristic of megakaryopoiesis is that cells are directed to accumulate nuclei while ceasing cell division. As a result, the mature MKs become polyploid with a ploidy number (N) ≥ 8 . Although

the relationship between polyploidization and released platelets remains unclear, it has been suggested that the polyploidy state is important to allow platelet release from mature MKs (Tavassoli 1980).

Polyploid MKs are stimulated by the BM microenvironment to migrate into blood vessels and protrude as proplatelets into bloodstream (Junt et al. 2007; Machlus and Italiano 2013). In combination with the BM microenvironment, MK migration and proplatelet formation are triggered by chemokines, including stromal derived factor-1 and fibroblast growth factor-4 (Avecilla et al. 2004). Finally, the shear force caused by blood flow facilitates the release of platelets from mature MKs.

Normally, the human body is programmed to maintain a sufficient platelet level. However, in certain conditions, including repeated chemotherapy, invasive surgery, or organ transplantation, platelet transfusion becomes necessary. Thrombocytopenia, platelet disorder caused by a low platelet count (less than 10^{10} platelets/L), is a life-threatening disease that can occur at any age. The disorder can be caused by multiple factors such as genetic mutation, drug treatment, autoimmune disease, BM failure, leukemia, and repeated-chemotherapy (Gresele 2008). As end-stage cancer typically requires chemotherapy for curative treatment, a growing number of cancer patients may lead to the increase in patients with thrombocytopenia.

Previously, the sole source of platelets for transfusion was direct isolation from healthy donors. Isolated platelets are commonly stored at room temperature to avoid the decrease in function that can arise at lower temperatures. Consequently, isolated platelets are viable for a maximum of 5–7 days (Gitz et al. 2012), making routine platelet isolation is the only option for maintaining a constant supply of platelets.

Recruitment of platelet donors is challenging especially in countries with declining population growth. Moreover, modern lifestyles have affected the willingness of individuals to dedicate time for platelet donation (Carter et al. 2011). Accordingly, relying platelet stock from human sources are unpredictable, as a shortage of platelet donor may occur at any time. The study of ex vivo platelet fabrication has been elucidated for decades and the functionality of produced platelets was confirmed based on in vivo and in vitro assays (Choi et al. 1995; Lu et al. 2011). Based on these viewpoints, the ex vivo platelet production offers a prominent alternative to human-isolated platelets for ensuring platelet supply.

Approaches to ex vivo platelet production

Natural and artificial materials have been developed as substitutes for human-isolated platelets. Table 1 summarizes the selected studies on the production of biomaterials with platelet-like characteristics.

Cell-based production

Hematopoietic stem cells

Initially, the approaches for generating platelet-substitute were performed by mimicking megakaryopoiesis in vitro. Platelets were initially generated in vitro by culturing HSCs in aplastic canine serum (Mazur et al. 1990), although TPO has been identified two decades ago (Odell et al. 1969). A subsequent study presented evidences that platelets produced from this system functioned in a similar manner to human-isolated platelets (Choi et al. 1995). Since then, the successful purification of TPO from serum (Kato et al. 1995) or plasma (Hoffman et al. 1985) and the synthesis of recombinant TPO (Bartley et al. 1994; Lok et al. 1994) have driven extensive studies on optimizing HSCs as platelet-producing cells in vitro.

HSCs, identified as $CD34^{+}$ cells, are typically isolated from BM (BM-derived $CD34^{+}$ cells) and cord blood (CB-derived $CD34^{+}$ cells). The availability of HSCs in the human body is scarce, with less than 0.1 % of all nucleated cells stored in BM (Pang et al. 2005). In recent years, $CD34^{+}$ cells can be collected from peripheral blood using drug stimulation (PB-derived $CD34^{+}$ cells). By administering granulocyte-stimulating factor, HSCs can be mobilized into peripheral blood and collected in a more convenient way.

CB-derived $CD34^{+}$ cells typically have a higher proliferation capacity, up to fivefold, than that of BM- or PB-derived $CD34^{+}$ cells (van den Oudenrijn et al. 2000). However, CB-derived $CD34^{+}$ cells have difficulty in achieving full maturation, as analyzed by the formation of polyploid MKs. After a week of TPO induction, as confirmed by the author (Nurhayati 2015), typically less than 10 % of CB-derived MKs are identified as polyploid cells while PB-derived cells produce about 20 % of polyploid MKs (Mattia et al. 2002; van den Oudenrijn et al. 2000) and BM-derived cells produce 10–15 % of polyploid MKs (Miyazaki et al. 2000). The formation of polyploid MKs is highly

Table 1 Selected reports on the production of biomaterials with platelet-like characteristics

Method	Source	Reported approach	Advantage	Disadvantage
Cell-based production	HSCs	Culturing HSCs in the presence of TPO and cytokines (Choi et al. 1995; Matsunaga et al. 2006)	Producing fully functional platelets	Low expansion Depending on HSC donor Loss of platelet activity during MK culture
	Mononuclear blood cells	Reprogramming blood cells into iPSCs and incorporating anti-apoptotic genes to create iMKCLs (Nakamura et al. 2014; Takayama et al. 2010)	Producing fully functional platelets	High cost Loss of platelet activity during MK culture
	Immortalized MKs	Culturing immortalized MKs established from leukemia patients, including CHRF-288-11 (Fugman et al. 1990), MEG01 (Schweinfurth et al. 2010), UT7/TPO (Komatsu et al. 1996), DAMI (Greenberg et al. 1988) cells, in the presence of inducer, such as TPO, valproic acid, and phorbol myristate acetate	Feasible for large-scale production Producing platelet-like particles	Low platelet quality Loss of platelet activity during MK culture
Synthetic material-based production	Polystyrene	Fabricating microparticles from polystyrene and then coating with platelet glycoprotein GPIb and vWF (Doshi et al. 2012)	Feasible for designing platelet properties Easy to control the quality	Lacking of major platelet features

correlated with platelet release (Mattia et al. 2002; Tavassoli 1980). Therefore, it is expected that PB-derived HSCs are more promising as platelet-producing cells ex vivo than BM- or CB-derived HSCs.

Induced pluripotent stem cells

The limited proliferation capacity and presence of donor-specific antigen are the major obstacles to establishing HSC-based platelet production. Accordingly, immortalized megakaryocytic cell lines (designated as iMKCLs) have been generated from blood cells or skin cells through induced pluripotent stem cell (iPSC) technology (Nakamura et al. 2014; Takayama et al. 2010). The incorporation of various anti-apoptotic genes, including *BM1*, *BCL-XL* and *c-MYC*, has successfully extended the proliferation capacity of iMKCLs for periods of up to 5 months (Nakamura et al. 2014). This method enables large-scale ex vivo platelet production using iMKCLs generated from patient cells (autologous platelet transfusion) on a clinically available scale, as developed by Megakaryon company (<http://www.megakaryon.com/en/>). Conventionally, establishment of a single iPSC clone costs 1300 USD (Beers et al.

2015) and complex nutritious media are required to maintain the cell pluripotency. The high cost of iPSC-based technology thus presents a real challenge to the mass production of platelet substitutes.

Immortalized MKs from leukemia patients

Immortalized MKs derived from leukemia patients offer a feasible strategy for producing platelet-like materials (Gandhi et al. 2005). UT7/TPO (Komatsu et al. 1996), MEG01 (Takeuchi et al. 1991), DAMI (Greenberg et al. 1988), and CHRF-288-11 (Jiang et al. 2002) cell lines are among the immortalized MKs that are able to generate platelet-like particles, as characterized by platelet glycoproteins, morphology and response to agonist stimulation. These cells can expand indefinitely and remain relatively stable in a long-term culture. Moreover, the cells are inducible in a single-step culture for producing platelet-like particles (Gandhi et al. 2005; Saito 1997), therefore the production costs are relatively inexpensive as compared with HSC-based or iPSC-based technologies. It should be noted that the immortalized MKs typically have low expressions of platelet glycoproteins or lack in them. Therefore, genetic modification and

optimization of culture conditions are necessary to improve the quality of platelets produced. Treatment with chemicals, such as valproic acid and TEMOSPho (2-(trimethylammonium)ethyl (R)-3-methoxy-3-oxo-2-stearamidopropyl phosphate), has been found to improve the functionality of platelet-like particles produced from MEG01 and K562 cells, respectively, as demonstrated by an increase in GPIb expression and ability to aggregate upon agonist stimulation (Limb et al. 2015; Schweinfurth et al. 2010).

Synthetic material-based production

In addition to cell-based platelet production, artificial platelet-like particles have been successfully generated from polystyrene-based materials. Polystyrene microparticles were stretched to form biconvex discoidal shape and then tailored with recombinant platelet glycoprotein vWF-A1 (Doshi et al. 2012). The artificial platelets were intended for drug delivery and shown to mimic the adhesion mechanism of natural platelets upon shear stress stimulation. Although these microparticles require major improvement to mimic the complex functional structure of natural platelets, the reported technique may enable the development of a more controllable technique that avoids undesirable platelet activation using cell-based technology, as described in section “[Challenges on scale-up of ex vivo platelet production](#)”.

Challenges on scale-up of ex vivo platelet production

The production of platelets was initially planned in HSCs because it has been widely accepted that functional platelets can be produced in vitro from this type of cells; however, several obstacles have impeded the scaling-up of this process. The major challenges to HSC-derived ex vivo platelet production are summarized in Table 2.

Limitation of HSCs

Low HSC expansion

HSCs have a limited expansion capacity outside of the human body. CB-derived HSCs can be expanded up to fivefold in vitro, however, these cells perform poorly

in the production of mature MKs (Miyazaki et al. 2000) or platelets (Mattia et al. 2002). PB-derived HSCs are preferable for generating platelets but the proliferation capacity is reduced once the cells are committed into megakaryopoiesis. Therefore, a multi-phase culture system was proposed to allow successive goals: extensive HSC expansion, optimized MK maturation, and promotion of platelet formation (Matsunaga et al. 2006; Panuganti et al. 2013). Nevertheless, this strategy is applicable only to a single platelet production process. As a consequence, repeated HSC isolation is required for initiating ex vivo platelet production.

The establishment of iMKCLs from blood cells offers a new approach to the prolonged expansion of platelet-producing cells. The cells can be cryopreserved and expanded for several months (Nakamura et al. 2014). Thus, the repeated cycles of platelet production are possible.

Low efficiency of megakaryopoiesis

Megakaryopoiesis ex vivo is less efficient than the in vivo process. Mature MKs can reach 128 N in the body and release thousands of platelets per mature MK (Deutsch and Tomer 2006) whereas mature MKs derived from an in vitro culture rarely attain 64 N and produce less than a hundred platelets per cell (Mattia et al. 2002). Several methods have been proposed to enhance megakaryopoiesis of HSCs, such as the addition of cytokine cocktails (Panuganti et al. 2013; van den Oudenrijn et al. 1999), chemical treatment (Avanzi et al. 2012; Giammona et al. 2006; Lannutti et al. 2005) and oxidative stress (Mostafa et al. 2000; Ojima et al. 2013). These methods can improve the in vitro megakaryopoiesis as confirmed by the increase in MK surface marker expression and the promoted polyploidization to up to 50 % of polyploid MKs after a week of culturing.

Treatment with chemicals including nicotinamide (Giammona et al. 2006), a Rho-Rock inhibitor (Y27632) (Lordier et al. 2008), and a Src inhibitor (SU6656) (Lannutti et al. 2005) was shown to be effective for promoting polyploidization of HSC-derived MKs. These chemicals are known to inhibit cytokinesis and thus restrict MK cell expansion (Avanzi et al. 2012). We found that the promoted polyploidization did not affect the platelet yield per MK in a culture of CHRF-derived MKs treated with a

Table 2 Typical challenges and proposed solutions to improve ex vivo platelet production

Target	Challenge	Proposed solution	Positive effect
Cell	Low HSC expansion	Three-stage culture to allow extensive proliferation, optimized MK maturation, and platelet release (Panuganti et al. 2013)	Increased quantity of MKs
		Establishment of iMKCLs (Nakagawa et al. 2013)	Extended proliferation capacity up to 5 months
	Low efficiency of megakaryopoiesis	Cytokines cocktails (TPO, interleukins, stem cell factor, etc.) (Panuganti et al. 2013; van den Oudenrijn et al. 1999)	Promoted megakaryopoiesis, as characterized from polyploidization and MK marker expression
		Chemical treatments (Nicotinamide, Y27632, SU6656) (Avanzi et al. 2012)	
Platelet	Low platelet yield	Oxygen tension (Mostafa et al. 2000), oxidative stress (Nurhayati et al. 2014; Ojima et al. 2013)	
		Shear stress caused from differential flows (Nakagawa et al. 2013)	Increased platelet yield
	Shedding of GPIb protein	Treatment with metalloproteinase inhibitor (Nishikii et al. 2008)	Reduced GPIb shedding in embryonic stem cell-derived platelets
	Premature platelet activation	Immediate platelet harvesting	Not reported

multi-kinase inhibitor, BMS-777607 (Nurhayati et al. 2015), suggesting that the number of MKs in culture was a dominant factor in determining the amount of released platelets. Accordingly, chemical treatment at a late stage of culturing is optimal to achieve the promoted polyploidization without compromising the cell expansion (Panuganti et al. 2013).

Low yield and quality of platelets

Naturally, MKs mature heterogeneously and the use of polyploidy inducers is beneficial for promoting polyploidization and maturation of MKs (Giammona et al. 2006; Nurhayati et al. 2015). Although the majority of cells can be artificially induced to form proplatelets by chemical treatment, the release of platelets from MKs does not occur simultaneously in a static culture condition, resulting in low platelet counts during the harvesting period (Norol et al. 1998). A bioreactor was therefore designed to mimic the shear stress caused by blood flow in vivo (Nakagawa et al. 2013). In this system, MKs were exposed to two differential flows, with a 60° in angle between the two flows. The use of the bioreactor was shown to improve platelet yield up to 3.6-fold, compared with static culture conditions (Nakagawa et al. 2013). By employing this technique, the requirement for seeded MK progenitors can be

reduced. To our knowledge, Matsunaga et al. (2006) reported the highest yields of platelet per seeded HSC, generating $1.26\text{--}1.68 \times 10^{11}$ platelets from 5×10^6 HSCs (amount of CB-derived HSCs per donor). Considering that a transfusion unit requires $3\text{--}5 \times 10^{11}$ platelets (Slichter 2006), the same number of seeded HSCs can produce one transfusion unit, meaning that multiple HSC donors are not required. This technique is promising for implementation in a large-scale culture with some modifications, especially after optimizing the flow contact area and combining the bioreactor with continuous platelet collection.

Circulating platelets remain inactive until exposed to agonists released from injured blood vessel. Under normal physiologic conditions, the resting state of platelets should be maintained so that blood clots are not formed at inappropriate sites. Endothelial cells play an important role in this process, particularly through the excretion of inhibitory agents such as nitric oxide, prostaglandins, and ectonucleotidases (Gresele 2008), and are therefore often used for layering purposes during MK culture. However, the use of endothelial cells is relatively inconvenient in a large-scale culture.

Without feeder cells, interaction between released platelets and excreted materials during in vitro culture

often induces premature platelet activation. It was reported that treatment with metalloproteinase inhibitor, GM6001, during MK culture could reduce the progress of platelet activation by inhibiting GPIb shedding (Nishikii et al. 2008); however, the inhibition of CD62P release from α -granule upon platelet activation has not yet been achieved. Although it was reported that culture-derived platelets remain functional, as evidenced from the ability to respond to agonist stimulation (Panuganti et al. 2013), the modification of culture conditions is necessary to optimize both of platelet yield and quality, to reach sufficient platelet quality for transfusion.

Prospect of ex vivo platelet production

Platelet production

In addition to low efficiency, production of HSC-derived platelets is limited by the existence of donor-specific human platelet antigens (HPAs), which can induce undesirable immune responses from repeated transfusion. Using iPSC-based technology, platelets can be expanded from autologous patient blood cells, thereby minimizing immune rejection after transfusion. Immortalized MKs from leukemia cells may also be a potential source for large-scale platelet production. Both of the platelet-producing cells are abundant and it is feasible to modify the cells for creating HPA-null or HPA-matched MKs. This technology may realize the universal platelet supply in the future. For these reasons, iPSC-derived and immortalized MKs are more promising sources for platelet production than HSCs.

Conventionally, ex vivo platelet generation is performed in a polystyrene dish or T-flask. Inspired by the BM environment, various modifications of culture vessel have been introduced to improve ex vivo megakaryopoiesis. A soft two-dimensional matrix, fabricated from collagen gel, has been reported to facilitate the proplatelet formation in MKs as a result of physical interactions between the cell membrane and matrix surface (Shin et al. 2011). Another study reported that a three-dimensional environment, created using porous polysaccharide-based hydrogels, could promote proplatelet formation up to threefold as compared with conventional culturing systems (Pietrzyk-Nivau et al. 2015). However, both studies

derived conclusions based on visual observation by microscopic analysis, without validation with universal platelet markers, such as GPIb or GPIIb/IIIa. Moreover, the promoted MK maturation by chemical treatment using blebbistatin, for example, can be reduced in a modified culture environment (Shin et al. 2011). Thus, the positive effect of surface environment on in vitro platelet production is undetermined yet.

The necessity of cell attachment during megakaryopoiesis remains unclear. HSC-derived MKs are non-adherent cells whereas iPSC-derived MKs are typically cultured in a feeder cell system. Nevertheless, a feeder-free system has been developed to facilitate the megakaryopoiesis of iPSC-derived MKs (Feng et al. 2014). It can be assumed that direct interaction of MKs with a vessel surface is not required to support megakaryopoiesis. Therefore, culture vessel can be expanded to a large-scale bioreactor (≥ 1 L) with consideration given to factors such as aeration and nutrient distribution.

Platelet separation

Platelets released into MK culture are often interspersed with cellular debris from dead cells. Commonly, platelets are separated from MKs using differential centrifugation (Matsunaga et al. 2006; Nakagawa et al. 2013), which may result in impurities. Moreover, this method is time-consuming and impracticable for large-scale culture. Recently, a feasible method was developed based on membrane filtration technique (Schlinker et al. 2015). MK cultures are pumped into spinning-membrane system and then, the MKs are retained by the membrane while the platelets pass through. This method can be further optimized to enable a continuous separation process for supporting a large-scale platelet bioreactor.

Good manufacturing practice (GMP) in ex vivo platelet production

GMP for pharmaceutical product, initiated by the Food and Drug Administration (FDA) of the United States, has been widely accepted as a basic guideline for producing cell-based medicine. Similar to other pharmaceutical products, GMP should be employed for producing clinical grade platelets, ensuring the quality and the safety of produced platelets. The GMP-

standards cover the materials, production process and safety testing of the final product (Unger et al. 2008).

HSCs are likely the most readily available GMP-grade cell source for platelet production. The cells have been clinically used, under GMP-standards, in post-chemotherapy patients. Isolation (Ohmine et al. 2011) and expansion (Bosse et al. 1997) of GMP-grade HSCs have been reported, ensuring the safety of HSCs for platelet production. In the case of iPSC-derived MKs, cells are commonly derived from HSCs (Takayama et al. 2010; Feng et al. 2014); therefore, the cell manipulation process is of critical importance in meeting GMP-standards. For iPSC-derived MKs, a tumorigenicity test should be performed and the use of non-integrating over integrating vectors is recommended (Ohmine et al. 2011). On the other hand, the use of immortalized MKs previously established from leukemia patients presents a significant challenge to meet GMP-standards. Based on FDA regulations, the use of cell lines derived from non-GMP sources is unacceptable unless the cells possess unreplaceable benefit to humans (Feigal et al. 2014). This condition indicates the importance of GMP-standards during the establishment of newly isolated immortalized MKs in the future.

Conventionally, *in vitro* platelet generation uses animal- or microbe-derived materials such as fetal bovine serum, feeder cells, antibiotics and growth factors/cytokines. GMP-grade antibiotics and growth factors/cytokines are readily available for clinical applications, while human-serum and serum replacements are considered to be GMP-grade alternatives to animal-based serum. Moreover, serum-free and feeder-free culture systems have been developed and have successfully generated functional platelets from iPSC-derived MKs (Feng et al. 2014).

A GMP-grade production process requires clean room facility for preparing materials, culturing cells and harvesting platelets. Direct contact with human should be minimized to avoid contamination and for this reason, various supporting devices have been developed. In a more advanced technology, automated culturing systems offer a minimal human handling, thus providing conditions that approach GMP-standards. Such systems have been developed by several companies, such as Cytogration (Brandel), SelecT (Automation Partnership), and AcCelerator (RTS Life Science International), capable of performing almost all operations in cell cultures such as cell inoculation,

medium change, cell harvesting, and analysis (Kempner and Felder 2002). The use of automated culturing system has also been reported to maintain iPSCs, considered to be highly sensitive cells, for 60 days in culture without loss of pluripotency (Konagaya et al. 2015). The culture system for platelet production does not much differ from other cell types, indicating that GMP-grade platelet production processes can be implemented.

Platelets differ from other human-derived cells in that they do not contain nuclei, and therefore have a low tumorigenic potential. However, safety testing is required before the produced platelets can be used clinically. In addition to tumorigenicity testing, basic safety testing on human-isolated platelets can become a reference for ensuring the quality and safety of *ex vivo* produced platelets.

In conclusion, recent findings related to MK culture suggest the possibility of producing clinical-grade platelets *ex vivo* in large-scale culture. Further developments of cell-culturing and platelet-harvesting technologies will facilitate improvements in quality and yield of *ex vivo* generated platelets.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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